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TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

DEX-0192

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/807200

INTERNATIONAL APPLICATION NO.  
PCT/US99/23764INTERNATIONAL FILING DATE  
18 October 1999PRIORITY DATE CLAIMED  
19 October 1998

TITLE OF INVENTION

Method of Diagnosing, Monitoring, Staging, Imaging and Treating Prostate Cancer

APPLICANT(S) FOR DO/EO/US

ALI, Shujath M. et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ has been transmitted by the International Bureau.
  - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). - **unexecuted**
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

## Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

- 1) Courtesy copy of International Application;
- 2) Copy of Written Opinion; and
- 3) Return Post card

"Express Mail" Label No. **EL718096873US**  
Date of Deposit **April 10, 2001**

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

By Deborah Ehret  
Typed Name: Deborah Ehret

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.53)

09/807200

INTERNATIONAL APPLICATION NO.

PCT/US99/23764

ATTORNEY'S DOCKET NUMBER

DEX-0192

21. The following fees are submitted:

**BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5) ) :**

- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... **\$1,000.00**
- ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... **\$860.00**
- ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... **\$710.00**
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... **\$690.00**
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... **\$100.00**

**ENTER APPROPRIATE BASIC FEE AMOUNT =****\$860.00**Surcharge of **\$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).**\$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	11 - 20 =	0	x \$18.00
Independent claims	6 - 3 =	3	x \$80.00

**\$0.00****\$240.00**Multiple Dependent Claims (check if applicable). ☐**\$0.00****TOTAL OF ABOVE CALCULATIONS =****\$1,100.00**Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). ☐**\$0.00****SUBTOTAL =****\$1,100.00**Processing fee of **\$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).**\$0.00****TOTAL NATIONAL FEE =****\$1,100.00**Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☐**\$0.00****TOTAL FEES ENCLOSED =****\$1,100.00**

Amount to be:	\$
refunded	
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☐ A check in the amount of \_\_\_\_\_ to cover the above fees is enclosed.**X Credit Card Payment form for \$1,100.00**☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \_\_\_\_\_ to cover the above fees.

A duplicate copy of this sheet is enclosed.

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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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REGISTRATION NUMBER

April 10, 2001

DATE

METHOD OF DIAGNOSING,  
MONITORING, STAGING, IMAGING AND TREATING PROSTATE CANCER

FIELD OF THE INVENTION

This invention relates, in part, to newly developed  
5 assays for detecting, diagnosing, monitoring, staging,  
prognosticating, imaging and treating cancers, particularly  
prostate cancer.

BACKGROUND OF THE INVENTION

Cancer of the prostate is the most prevalent malignancy  
10 in adult males, excluding skin cancer, and is an increasingly  
prevalent health problem in the United States. In 1996, it  
was estimated that 41,400 deaths would result from this  
disease in the United States alone, indicating that prostate  
cancer is second only to lung cancer as the most common cause  
15 of death in the same population. If diagnosed and treated  
early, when the cancer is still confined to the prostate, the  
chances of cure is significantly higher.

Treatment decisions for an individual are linked to the  
stage of prostate cancer present in that individual. A common  
20 classification of the spread of prostate cancer was developed  
by the American Urological Association (AUA). The AUA system  
divides prostate tumors into four stages, A to D. Stage A,  
microscopic cancer within prostate, is further subdivided into  
sub-stages A1 and A2. Sub-stage A1 is a well-differentiated  
25 cancer confined to one site within the prostate. Treatment  
is generally observation, radical prostatectomy, or radiation.  
Sub-stage A2 is a moderately to poorly differentiated cancer  
at multiple sites within the prostate. Treatment is radical  
prostatectomy or radiation. Stage B, palpable lump within the

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prostate, is also further subdivided into sub-stages B1 and B2. In sub-stage B1, the cancer forms a small nodule in one lobe of the prostate. In sub-stage B2, the cancer forms large or multiple nodules, or occurs in both lobes of the prostate.

5 Treatment for sub-stages B1 and B2 is either radical prostatectomy or radiation. Stage C is a large cancer mass involving most or all of the prostate and is also further subdivided into two stages. In sub-stage C1, the cancer forms a continuous mass that may have extended beyond the prostate.

10 In sub-stage C2, the cancer forms a continuous mass that invades the surrounding tissue. Treatment for both these sub-stages is radiation with or without drugs to address the cancer. The fourth stage, Stage D is metastatic cancer and is also subdivided into two sub-stages. In sub-stage D1, the

15 cancer appears in the lymph nodes of the pelvis. In sub-stage D2, the cancer involves tissues beyond lymph nodes. Treatment for both of these sub-stages is systemic drugs to address the cancer as well as pain.

However, current prostate cancer staging methods are

20 limited. As many as 50% of prostate cancers initially staged as A2, B, or C are actually stage D, metastatic. Discovery of metastasis is significant because patients with metastatic cancers have a poorer prognosis and require significantly different therapy than those with localized cancers. The five

25 year survival rates for patients with localized and metastatic prostate cancers are 93% and 29%, respectively.

Accordingly, there is a great need for increasingly sensitive methods for diagnosing and staging of prostate cancer in a human patient to determine whether or not such

30 cancer has metastasized and for monitoring the progress of a cancer which has not metastasized for the onset of metastasis.

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In the present invention, methods are provided for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating prostate cancer via the cancer specific gene referred to herein as CSG. CSG refers, among other things, to native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted herein as SEQ ID NO:2. In the alternative, what is meant by CSG as used herein, means the native mRNA encoded by the gene comprising the polynucleotide sequence of SEQ ID NO:1 or levels of the gene comprising the polynucleotide sequence of SEQ ID NO:1.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

#### SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of prostate cancer by analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein a change in levels of

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CSG in the patient versus the normal human control is associated with prostate cancer.

Further provided is a method of diagnosing metastatic prostate cancer in a patient having prostate cancer which is not known to have metastasized by identifying a human patient suspected of having prostate cancer that has metastasized; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in CSG levels in the patient versus the normal human control is associated with prostate cancer which has metastasized.

Also provided by the invention is a method of staging prostate cancer in a human which has such cancer by identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Further provided is a method of monitoring prostate cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in

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preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

5 Further provided is a method of monitoring the change in stage of prostate cancer in a human having such cancer by looking at levels of CSG in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissues, or  
10 bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is  
15 associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Further provided are antibodies targeted against CSG or fragments of such antibodies which can be used to detect or  
20 image localization of CSG in a patient for the purpose of detecting or diagnosing a disease or condition. Such antibodies can be polyclonal, monoclonal, or omnichlonal or prepared by molecular biology techniques. The term "antibody", as used herein and throughout the instant  
25 specification is also meant to include aptamers and single-stranded oligonucleotides such as those derived from an in vitro evolution protocol referred to as SELEX and well known to those skilled in the art. Antibodies can be labeled with a variety of detectable labels including, but not limited to,  
30 radioisotopes and paramagnetic metals. These antibodies or fragments thereof can also be used as therapeutic agents in

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the treatment of diseases characterized by expression of CSG. In therapeutic applications, the antibody can be used without or with derivatization to a cytotoxic agent such as a radioisotope, enzyme, toxin, drug or a prodrug.

5 Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of  
10 the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

#### 15 DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging and prognosticating cancers by comparing levels of CSG in a human patient with those of  
20 CSG in a normal human control. What is meant by levels of CSG as used herein, means levels of the native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted herein as SEQ ID NO:2. The native protein  
25 being detected, may be whole, a breakdown product, a complex of molecules or chemically modified. In the alternative, what is meant by levels of CSG as used herein, means levels of the native mRNA encoded by the gene comprising the polynucleotide sequence of SEQ ID NO:1 or levels of DNA comprising the  
30 polynucleotide sequence of SEQ ID NO:1. Such levels are



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preferably measured in at least one of cells, tissues and/or  
bodily fluids, including determination of normal and abnormal  
levels. Thus, for instance, a diagnostic assay in accordance  
with the invention for diagnosing over-expression of CSG  
5 protein compared to normal control bodily fluids, cells, or  
tissue samples may be used to diagnose the presence of  
prostate cancer.

All the methods of the present invention may optionally  
include measuring the levels of other cancer markers as well  
10 as CSG. Other cancer markers, in addition to CSG, useful in  
the present invention will depend on the cancer being tested  
and are known to those of skill in the art.

#### ***Diagnostic Assays***

The present invention provides methods for diagnosing the  
15 presence of prostate cancer by analyzing for changes in levels  
of CSG in cells, tissues or bodily fluids compared with levels  
of CSG in cells, tissues or bodily fluids of preferably the  
same type from a normal human control, wherein an increase in  
levels of CSG in the patient versus the normal human control  
20 is associated with the presence of prostate cancer.

Without limiting the instant invention, typically, for  
a quantitative diagnostic assay a positive result indicating  
the patient being tested has cancer is one in which cells,  
tissues or bodily fluid levels of the cancer marker, such as  
25 CSG, are at least two times higher, and most preferably are  
at least five times higher, than in preferably the same cells,  
tissues or bodily fluid of a normal human control.

The present invention also provides a method of  
diagnosing metastatic prostate cancer in a patient having  
30 prostate cancer which has not yet metastasized for the onset  
of metastasis. In the method of the present invention, a

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human cancer patient suspected of having prostate cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art.

5 In the present invention, determining the presence of CSG levels in cells, tissues or bodily fluid, is particularly useful for discriminating between prostate cancer which has not metastasized and prostate cancer which has metastasized. Existing techniques have difficulty discriminating between  
10 prostate cancer which has metastasized and prostate cancer which has not metastasized. However, proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels measured in such cells, tissues or bodily fluid is CSG.  
15 Measured levels of CSG are compared with levels of CSG in preferably the same cells, tissue or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just CSG in serum, this level is preferably compared with the level of CSG in serum of a normal human  
20 patient. An increase in CSG levels in the patient versus the normal human control is associated with prostate cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating  
25 the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily  
30 fluid of a normal patient.

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Normal human control as used herein includes a human patient without cancer and/or non cancerous samples from the patient; in the methods for diagnosing or monitoring for metastasis, normal human control may preferably also include 5 samples from a human patient that is determined by reliable methods to have prostate cancer which has not metastasized.

**Staging**

The invention also provides a method of staging prostate cancer in a human patient. The method comprises identifying 10 a human patient having such cancer and analyzing a sample of cells, tissues or bodily fluid from such human patient for CSG. The measured CSG levels in the patient are then compared with levels of CSG in preferably the same cells, tissues or 15 bodily fluid type of a normal human control, wherein an increase in CSG levels in the human patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG (but still increased over true normal levels) is associated with a cancer which is regressing or in remission.

**20 Monitoring**

Further provided is a method of monitoring prostate cancer in a human patient having such cancer for the onset of metastasis. The method comprises identifying a human patient having prostate cancer that is not known to have metastasized; 25 periodically analyzing cells, tissues or bodily fluid from such human patient for CSG; comparing the CSG levels in such cells, tissues or bodily fluid with levels of CSG in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in CSG levels in the 30 human patient versus the normal human control is associated

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with a cancer which has metastasized. In this method, normal human control samples may also include prior patient samples.

Further provided by this invention is a method of monitoring the change in stage of prostate cancer in a human patient having such cancer. The method comprises identifying a human patient having prostate cancer; periodically analyzing cells, tissues or bodily fluid from such human patient for CSG; comparing the CSG levels in such cells, tissues or bodily fluid with levels of CSG in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in CSG levels in the human patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of CSG is associated with a cancer which is regressing in stage or in remission. In this method, normal human control samples may also include prior patient samples.

Monitoring patients for onset of metastasis is periodic and preferably done on a quarterly basis. However, monitoring may be performed more or less frequently depending on the cancer, the particular patient, and the stage of the cancer.

#### **Assay Techniques**

Assay techniques that can be used to determine levels of gene expression (including protein levels), such as CSG of the present invention, in a sample derived from a patient are well known to those of skill in the art. Such assay methods include, without limitation, radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses, ELISA assays and proteomic approaches: two-dimensional gel electrophoresis (2D electrophoresis) and non-gel based approaches such as mass

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spectrometry or protein interaction profiling. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids.

An ELISA assay initially comprises preparing an antibody, 5 if not readily available from a commercial source, specific to CSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to CSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or 10 enzymatic reagent. For example, detectable agents such as horseradish peroxidase enzyme and alkaline phosphatase are routinely used in these types of assays.

To carry out the ELISA, antibody specific to CSG is incubated on a solid support, e.g. a polystyrene dish, that 15 binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time CSG binds to the specific antibody attached to the polystyrene 20 dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to CSG and linked to a detectable reagent such as horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to CSG. Unattached reporter 25 antibody is then washed out. Reagents for detection of peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase linked to CSG antibodies produces a colored reaction product. The amount of color developed in a given time period is proportional to 30 the amount of CSG protein present in the sample. Quantitative

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results typically are obtained by reference to a standard curve.

A competition assay can also be employed wherein antibodies specific to CSG are attached to a solid support. 5 Labeled CSG and a sample derived from the host are then passed over the solid support. The amount of label detected which is attached to the solid support can be correlated to a quantity of CSG in the sample.

Nucleic acid methods can be used to detect CSG mRNA as 10 a marker for prostate cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse- 15 transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse 20 transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of 25 cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e. gridding) can be used to detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the CSG gene is fixed 30 to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or

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plastic. At least a portion of the DNA encoding the CSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA isolated from the tissue of interest.

5 Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including, but not limited to, radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene  
10 expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.

15 Of the proteomic approaches, 2D electrophoresis is a technique well known to those in the art. Isolation of individual proteins from a sample such as serum is accomplished using sequential separation of proteins by different characteristics usually on polyacrylamide gels.  
20 First, proteins are separated by size using an electric current. The current acts uniformly on all proteins so that smaller proteins move farther on the gel than larger proteins. The second dimension applies a current perpendicular to the first and separates proteins not on the basis of size but on  
25 the specific electric charge carried by each protein. Since no two proteins with different sequences are identical on the basis of both size and charge, the result of a 2D separation is a square gel in which each protein occupies a unique spot. Analysis of the spots with chemical or antibody probes or  
30 subsequent protein microsequencing can reveal the relative

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abundance of a given protein and the identity of the proteins in the sample.

The above tests can be carried out on samples derived from a variety of cells, bodily fluids and/or tissue extracts including both homogenates or solubilized tissue obtained from a human patient. Tissue extracts can be obtained from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva or any other bodily secretion or derivative thereof. By blood it is meant to include whole blood, plasma, serum or any derivative of blood.

#### *In Vivo Antibody Use*

Antibodies which specifically bind to CSG can also be used in vivo in patients suspected of suffering from prostate cancer. Specifically, antibodies which specifically bind a CSG can be injected into a patient suspected of having prostate cancer for diagnostic and/or therapeutic purposes. The preparation and use of antibodies for *in vivo* diagnosis is well known in the art. For example, antibody-chelators labeled with Indium-111 have been described for use in the radioimmunosciintographic imaging of carcinoembryonic antigen expressing tumors (Sumerdon et al. Nucl. Med. Biol. 1990 17:247-254). In particular, these antibody-chelators have been used in detecting tumors in patients suspected of having recurrent colorectal cancer (Griffin et al. J. Clin. Onc. 1991 9:631-640). Antibodies with paramagnetic ions as labels for use in magnetic resonance imaging have also been described (Laufer, R.B. Magnetic Resonance in Medicine 1991 22:339-342). Antibodies directed against CSG can be used in a similar manner. Labeled antibodies which specifically bind CSG can be injected into patients suspected of having prostate



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cancer for the purpose of diagnosing or staging of the disease status of the patient. The label used will be selected in accordance with the imaging modality to be used. For example, radioactive labels such as Indium-111, Technetium-99m or  
5 Iodine-131 can be used for planar scans or single photon emission computed tomography (SPECT). Positron emitting labels such as Fluorine-19 can be used in positron emission tomography. Paramagnetic ions such as Gadolinium (III) or Manganese (II) can be used in magnetic resonance imaging  
10 (MRI). Localization of the label permits determination of the spread of the cancer. The amount of label within an organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue.

For patients diagnosed with prostate cancer, injection  
15 of an antibody which specifically binds CSG can also have a therapeutic benefit. The antibody may exert its therapeutic effect alone. Alternatively, the antibody can be conjugated to a cytotoxic agent such as a drug, toxin or radionuclide to enhance its therapeutic effect. Drug monoclonal antibodies  
20 have been described in the art for example by Garnett and Baldwin, Cancer Research 1986 46:2407-2412. The use of toxins conjugated to monoclonal antibodies for the therapy of various cancers has also been described by Pastan et al. Cell 1986 47:641-648. Yttrium-90 labeled monoclonal antibodies have  
25 been described for maximization of dose delivered to the tumor while limiting toxicity to normal tissues (Goodwin and Meares Cancer Supplement 1997 80:2675-2680). Other cytotoxic radionuclides including, but not limited to Copper-67, Iodine-131 and Rhenium-186 can also be used for labeling of  
30 antibodies against CSG.

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Antibodies which can be used in these *in vivo* methods include polyclonal, monoclonal and omniconal antibodies and antibodies prepared via molecular biology techniques. Antibody fragments and aptamers and single-stranded  
5 oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art can also be used.

The present invention is further described by the following examples. These examples are provided solely to  
10 illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

#### EXAMPLES

15 The examples are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as  
20 Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

#### Example 1

##### Suppression subtractive hybridization (CLONTECH PCR-SELECT)

25 CLONTECH PCR-SELECT is a PCR based subtractive hybridization method designed to selectively enrich for cDNAs corresponding to mRNAs differentially expressed between two mRNA populations (Diatchenko et al, *Proc. Natl. Acad. Sci. USA*, Vol. 93, pp. 6025-6030, 1996). In this method,  
30 differentially expressed mRNAs are enriched based on a selective amplification. cDNA is prepared from the two mRNA

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populations which are to be compared. These include the Tester population which is a cDNA population in which the differentially expressed messages are sought and the Driver population which is a cDNA population in which the differentially expressed transcripts are absent or low. The tester sample is separated in two parts and different PCR adapters are ligated to the 5' ends. Each tester is separately annealed to excess driver in the first annealing and then pooled and again annealed in the second annealing to excess driver. During the first annealing, sequences common to both populations anneal. Additionally the concentration of high and low abundance messages are normalized since annealing is faster for abundant molecules due to the second order kinetics of hybridization. During the second annealing cDNAs unique or overabundant to the tester can anneal together. Such molecules have different adapters at their ends. The addition of additional driver during the second annealing enhances the enrichment of the desired differentially expressed sequences. During subsequent PCR, molecules that have different adapters at each end amplify exponentially. Molecules which have identical adapters, or adapters at only one end, or no adapters (driver sequences) either do not amplify or undergo linear amplification. The end result is enrichment for cDNAs corresponding to differentially expressed messages unique to the tester or up regulated in the tester.

This technique was used to identify transcripts unique to cancer tissues or messages overexpressed in the cancer process. To do this, pairs of samples isolated from a cancer tissue were used as the "tester", and non-cancer tissue as the "driver". The non-cancer "driver" can be from the same

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individual and tissue as the tumor (Matched). Alternatively, the "driver" can be from a different individual but the same tissue as the tumor sample (unmatched). In some cases, the "driver" comprises mixtures of cDNAs derived from non-cancer  
5 tissues different from the cancer tissue. This approach allows the identification of transcripts whose expression is specific or up-regulated in the cancer tissue. Such transcripts may or may not be cancer specific in their expression.

10 Subtractive hybridization was carried out using as "tester" a mixture of three RNAs from human adenocarcinomas and as "driver" a mixture of RNAs from five human normal tissues (spleen, pancreas, heart, kidney and small intestine).

The subtracted mixture was cloned and two hundred clones  
15 were sequenced. One of the sequences matched Incyte clone ID 3966820. The electronic Northern for this clone showed the highest number of ESTs came from prostate, compared with other tissues (prostate 107 followed by uterus with 20).

The PCR-select clone detected a transcript of 1.9 kb by  
20 hybridization in Northern blots. Amongst 17 tissues tested prostate showed the highest abundance for this transcript.

#### Example 2: Relative Quantitation of Gene Expression

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'- 3'  
25 nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected

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by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and  
5 normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were  
10 used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of the target gene  
15 were evaluated for every sample in normal and cancer tissues. Total RNA was extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was  
20 done using primers and Taqman probes specific to each target gene. The results were analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

25 Real-Time quantitative PCR was done using the following primers:

pro108 Reverse GCCTTCAGCCGTGGGTAGT (SEQ ID NO:3)

pro108 Forward GACAGCGGCTTCACCTTCTC (SEQ ID NO:4)

The absolute numbers depicted in Table 1 are relative  
30 levels of expression of the CSG referred to herein as pro108 (SEQ ID NO:1) in 11 normal different tissues. All the values

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are compared to normal pancreas (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

5 Table 1: Relative Levels of CSG Pro108 Expression in Pooled Samples

	<b>Tissue</b>	<b>NORMAL</b>
	Colon Ascending	4.56
	Kidney	0.78
10	Liver	5.66
	Ovary	41.07
	Small Intestine	0.78
	Spleen	3.29
	Stomach	11.39
15	Testis	2.04
	Uterus	15.56
	Pancreas	1.00
	Prostate	2.32

The relative levels of expression in Table 1 show the  
20 highest mRNA expression in ovary (41.07) and uterus (15.56), two female specific tissues. Except for stomach (11.39) that shows high levels of mRNA for pro108, the other tissues including prostate show comparable low levels of expression.

The absolute numbers in Table 1 were obtained analyzing  
25 pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 2.

The absolute numbers depicted in Table 2 are relative  
30 levels of expression of the CSG pro108 in 70 pairs of matching samples. All the values are compared to normal pancreas (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

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Unmatched samples were used for prostatitis (prostatitis 1 and 2) and Benign Prostate Hyperplasia (BPH 1 to 6).

**Table 2: Relative Levels of CSG Pro108 Expression in Individual Samples**

	Sample ID	Tissue	Cancer	Benign Diseases	Normal Adjacent Tissue
5	Pro12B	Prostate 1	253.40		7.70
	Pro78XB	Prostate 2	1020.46		96.67
	Pro84XB	Prostate 3	2055.11		70.52
	Pro101XB	Prostate 4	872.61		218.58
10	Pro91X	Prostate 5	539.32		195.36
	Pro13XB	Prostate 6	0.60		0.49
	Pro23B	Prostate 7	929.30		747.00
	Pro90XB	Prostate 8	160.50		21.00
	Pro18XB	Prostate 9	45.63		31.49
15	Pro20XB	Prostate 10	312.50		20.00
	Pro34B	Prostate 11	1351.18		142.02
	Pro65XB	Prostate 12	1305.15		8.37
	Pro69XB	Prostate 13	486.50		14.50
	Pro10R	Prostatitis 1		122.45	
20	Pro20R	Prostatitis 2		83.38	
	Pro258BPH	BPH 1		39.33	
	Pro263CBPH	BPH 2		451.98	
	Pro267ABPH	BPH 3		90.78	
	Pro271ABPH	BPH 4		5.21	
25	Pro460ZBPH	BPH 5		15.64	
	ProC032BPH	BPH 6		46.85	
	Bld32XK	Bladder 1	2.86		3.88
	Bld46XK	Bladder 2	15.62		1.45

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	Bld66X	Bladder 3	32.67		19.90
	BldTR14	Bladder 4	330.60		235.50
	ClnAS67	Colon 1	71.26		0.51
	ClnRC01	Colon 2	5.08		25.90
5	ClnSG45	Colon 3	19.09		6.61
	ClnSG67	Colon 4	65.12		20.61
	ClnTX01	Colon 5	116.97		6.25
	ClnB34	Colon 6	3.98		2.45
	ClnB56	Colon 7	1.98		1.41
10	ClnC9XR	Colon 8	6.88		2.00
	ClnCXGA	Colon 9	2.25		2.62
	ClnAS89	Colon 10	77.44		2.39
	ClnTX67	Colon 11	167.73		11.51
	End10479	Endometrium 1	7.63		26.67
15	End8911	Endometrium 2	16.19		64.98
	End8963	Endometrium 3	12.14		65.89
	End5XA	Endometrium 4	99.39		23.51
	End65RA	Endometrium 5	6.94		16.06
	Kid109XD	Kidney 1	65.57		41.93
20	Kid10XD	Kidney 2	39.67		9.71
	Kid11XD	Kidney 3	12.68		1.62
	Kid126XD	Kidney 4	43.71		7.59
	Kid12XD	Kidney 5	2.50		70.80
	Kid5XD	Kidney 6	2.11		74.29
25	Kid6XD	Kidney 7	19.36		0.72
	Liv42X	Liver 1	39.00		6.40
	Liv15XA	Liver 2	9.23		4.59
	Liv94XA	Liver 3	7.07		8.09

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	LngAC66	Lung 1	47.30		16.20
	LngBR94	Lung 2	106.50		3.40
	LngLC109	Lung 3	9.60		34.10
	LngLC71	Lung 4	70.30		64.00
5	LngSQ56	Lung 5	12.40		53.80
	LngSQ79	Lung 6	108.40		77.20
	Lng60XL	Lung 7	0.55		1.47
	Lng75XC	Lung 8	2.34		3.11
	LngC17X	Lung 9	52.96		31.71
10	LngAC69	Lung 10	654.84		42.67
	LngC20X	Lung 11	2.82		0.22
	Mam47XP	Mammary Gland 1	3.40		1.89
	Mam82XI	Mammary Gland 2	33.29		4.44
	MamB011X	Mammary Gland 3	10.61		1.50
15	MamA06X	Mammary Gland 4	14.62		1.55
	Ovr	Ovary	10.00		8.00
	Pan77X	Pancreas 1	91.27		24.50
	Pan71XL	Pancreas 2	1.55		1.30
	Pan82XP	Pancreas 3	5.49		7.53
20	Pan92X	Pancreas 4	1069.00		688.40
	StoAC99	Stomach 1	50.70		18.60
	StoTA73	Stomach 2	37.70		130.30
	SmI21XA	Small Intestine 1	12.42		5.31
	SmIH89	Small Intestine 2	23.26		2.63
25	Tst39X	Testis	27.95		7.01

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Utr85XU	Uterus 1	3.30		4.91
Utr23XU	Uterus 2	44.48		29.04

Table 2 shows the results for the analysis of 148 samples distributed in 14 different tissue types. Tables 1 and 2 represent a combined total of 159 samples in 16 different tissue types.

In the analysis of matching samples, the higher levels of expression were in prostate, showing a high degree of tissue specificity for prostate tissue. Of all the samples different from prostate which were analyzed (57 matching samples), only a few samples (lung 10 and pancreas 4) showed an expression comparable or higher than the median for the mRNA expression in prostate cancer (median: 539.32).

In addition, the level of mRNA expression in cancer samples was compared with the isogenic normal adjacent tissue from the same individual. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 2 shows overexpression of the CSG pro108 in 13 of 13 prostate cancer tissues compared with their respective normal adjacent (prostate samples #1 to #13). Thus, there was overexpression in the cancer tissue for 100% of the prostate matching samples tested.

Altogether, the level of tissue specificity, plus the mRNA overexpression in 100% of the prostate matching samples tested are indicative of the CSG pro108 being a diagnostic marker for prostate cancer.

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What is claimed is:

1. A method for diagnosing the presence of prostate cancer in a patient comprising:

(a) measuring levels of CSG in cells, tissues or bodily fluids in a patient; and

(b) comparing the measured levels of CSG with levels of CSG in cells, tissues or bodily fluids from a normal human control, wherein a change in measured levels of CSG in said patient versus normal human control is associated with the presence of prostate cancer.

2. A method of diagnosing metastases of prostate cancer in a patient comprising:

(a) identifying a patient having prostate cancer that is not known to have metastasized;

(b) measuring CSG levels in a sample of cells, tissues, or bodily fluid from said patient; and

(c) comparing the measured CSG levels with levels of CSG in cells, tissue, or bodily fluid of a normal human control, wherein an increase in measured CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

3. A method of staging prostate cancer in a patient having prostate cancer comprising:

(a) identifying a patient having prostate cancer;

(b) measuring CSG levels in a sample of cells, tissue, or bodily fluid from said patient; and

(c) comparing measured CSG levels with levels of CSG in cells, tissues, or bodily fluid of a normal human control, wherein an increase in measured CSG levels in said patient

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versus the normal human control is associated with a cancer which is progressing and a decrease in the measured CSG levels is associated with a cancer which is regressing or in remission.

5        4. A method of monitoring prostate cancer in a patient for the onset of metastasis comprising:

(a) identifying a patient having prostate cancer that is not known to have metastasized;

10        (b) periodically measuring levels of CSG in samples of cells, tissues, or bodily fluid from said patient; and

15        (c) comparing the periodically measured CSG levels with levels of CSG in cells, tissues, or bodily fluid of a normal human control, wherein an increase in any one of the periodically measured CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

5. A method of monitoring a change in stage of prostate cancer in a patient comprising:

(a) identifying a patient having prostate cancer;

20        (b) periodically measuring levels of CSG in cells, tissues, or bodily fluid from said patient; and

25        (c) comparing the periodically measured CSG levels with levels of CSG in cells, tissues, or bodily fluid of a normal human control, wherein an increase in any one of the periodically measured CSG levels in the patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease is associated with a cancer which is regressing in stage or in remission.



Docket No.  
DEX-0192

# Declaration and Power of Attorney For Patent Application

## English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**Method of Diagnosing, Monitoring, Staging, Imaging and Treating Prostate Cancer**

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 18 October 1999 as United States Application No. or PCT International

Application Number PCT/US99/23764

and was amended on \_\_\_\_\_

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/104,741

19 October 1998

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)

(Filing Date)

(Status)  
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)  
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)  
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*



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